

CLAIMS

What is claimed is:

1. A recombinant polypeptide that is or contains a KDPGal aldolase having at least one of the mutations: X10V, X28L or X28M, X42T, X85A, X154F, or X196I.
2. The recombinant polypeptide of Claim 1, wherein said KDPGal aldolase has at least one of the mutations: I10V, V28L or V28M, S42T, V85A, V154F, or F196I.
3. The recombinant polypeptide of Claim 1, wherein said KDPGal aldolase has the amino acid sequence of any of SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6, and said at least one mutation is a mutation thereto.
4. The recombinant polypeptide of Claim 1, wherein said KDPGal aldolase has no mutation that is X70L.
5. The recombinant polypeptide of Claim 1, wherein said KDPGal aldolase has an amino acid sequence at least 50% homologous to that of any of SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6, and said at least one mutation is a mutation thereto.
6. The recombinant polypeptide of Claim 1, wherein said KDPGal aldolase has an amino acid sequence about 190 to about 215 residues in length.
7. The recombinant polypeptide of Claim 1, wherein said KDPGal aldolase has an amino acid sequence about 200 to about 210 residues in length.
8. The recombinant polypeptide of Claim 1, wherein said KDPGal aldolase has an amino acid sequence about 205 residues in length.
9. The recombinant polypeptide of Claim 1, wherein said KDPGal aldolase has the amino acid sequence of a native bacterial KDPGal aldolase that has been mutated to contain said at least one mutation.
10. The recombinant polypeptide of Claim 9, wherein said native bacterial KDPGal aldolase is native to a member of the proteobacteria.
11. The recombinant polypeptide of Claim 10, wherein said native bacterial KDPGal aldolase is native to a member of any one of the genera *Agrobacterium*, *Bradyrhizobium*, *Brucella*, *Caulobacter*, *Escherichia*, *Klebsiella*, *Ralstonia*, *Salmonella*, and *Sinorhizobium*.

12. Nucleic acid encoding a recombinant polypeptide according to any one of Claims 1-11.
13. The nucleic acid according to Claim 12, wherein the coding sequence thereof that encodes the KDPGal aldolase of the polypeptide has a nucleotide sequence more than 80% homologous to that of any of SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:5.
14. The nucleic acid according to Claim 12, wherein said nucleic acid is at least one nucleic acid vector.
15. The nucleic acid according to Claim 14, wherein said vector is at least one plasmid.
16. An enzymatic pathway capable of converting pyruvate and D-erythrose 4-phosphate (E4P) into 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP), said pathway including at least one KDPGal aldolase.
17. The enzymatic pathway of Claim 16, further comprising at least one DHQ synthase, said pathway being capable of synthesizing 3-dehydroquinate (DHQ) from DAHP.
18. The enzymatic pathway of Claim 17, further comprising at least one DHQ dehydratase, said pathway being capable of synthesizing 3-dehydroshikimate (DHS) from DHQ.
19. The enzymatic pathway of Claim 18, further comprising at least one shikimate dehydrogenase, said pathway being capable of synthesizing shikimate from DHS.
20. A method for the production of shikimate or a shikimate intermediate comprising (1) providing a recombinant cell containing nucleic acid encoding at least one KDPGal aldolase and at least one DHQ synthase, from which nucleic acid said cell can express those enzymes, and (2) growing said cell in a medium under conditions in which it expresses them; and (3) optionally, recovering at least one of DAHP, DHQ, DHS, or a further derivative thereof, from said medium or from said cell.
21. The method of Claim 20, wherein the shikimate intermediate is at least one of DAHP, DHQ, or DHS.
22. The method of Claim 20, wherein said recombinant cell, when grown under said conditions, expresses at least one recombinant transketolase or at least one recombinant transaldolase.

23. A method for converting pyruvate and E4P to DAHP, comprising contacting a KDPGal aldolase with a solution containing pyruvate and E4P.

24. The method of Claim 23, wherein said method further includes contacting said DAHP with a DHQ synthase, thereby forming DHQ.

5 25. The method of Claim 24, wherein said method further includes contacting said DHQ with a DHQ dehydratase, thereby forming 3-dehydroshikimate.

26. The method of to any one of Claims 23-25, wherein said method is performed within a recombinant cell.

10 27. The method of Claim 26, wherein said host cell was produced by transforming the cell with nucleic acid encoding at least one of a KDPGal aldolase or a DHQ synthase.

28. The method of Claim 26, wherein said recombinant cell contains at least one recombinant transketolase or at least one recombinant
15 transaldolase.

29. Use of a recombinant KDPGal aldolase to produce DAHP from pyruvate and E4P.

30. The use according to Claim 19, wherein said use further includes use of a recombinant DHQ synthase to produce DHQ from said
20 DAHP.

31. A process for preparing a recombinant cell capable of expressing a KDPGal aldolase, and of converting pyruvate and E4P to DAHP by action thereof, comprising:

A) providing a host cell capable of synthesizing pyruvate and E4P,
25 B) providing a vector containing a polynucleotide from which said host cell can express a KDPGal aldolase, and
C) transforming said cell with said vector to produce a transformed cell, and, optionally, thereafter expressing said KDPGal aldolase, whereupon said transformed cell converts pyruvate and E4P to DAHP.

30 32. The process according to Claim 31, wherein said KDPGal aldolase has an amino acid sequence at least 50% homologous to that of any one of SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6.

33. The process according to Claim 32, wherein said KDPGal aldolase has at least one of the mutations: X10V, X28L or X28M, X42T, X85A, X154F, or X196I.

34. A recombinant cell prepared by the process according to any
5 one of Claims 31-33.

35. The cell according to Claim 34, wherein said cell is a walled cell.

36. The cell according to Claim 35, wherein said cell is a bacterial cell.

37. The cell according to Claim 34, wherein said cell is an an
10 *aroFGH* cell.

38. A process for preparing at least one of DAHP or a derivative thereof, said process including the steps of:

1) providing

- (A) a supply of E4P and pyruvate,
- 15 (B) a KDPGal aldolase, and optionally a DHQ synthase,
- (C) an aqueous medium,

2) contacting in said medium, said KDPGal aldolase with said E4P and said pyruvate under conditions in which said KDPGal aldolase can catalyze the formation of DAHP from the E4P and pyruvate, and optionally contacting said
20 DAHP with said DHQ synthase under conditions in which said DHQ synthase can catalyze the formation of 3-dehydroquininate from the DAHP;
3) optionally recovering at least one of DAHP, DHQ, DHS, or a further derivative thereof, from said medium.

39. A kit containing a KDPGal aldolase preparation, with instructions
25 for the use thereof to convert pyruvate and E4P to DAHP, and optionally with instructions for the conversion of said DAHP to at least one derivative thereof.

40. A kit containing a cell capable of expressing a KDPGal aldolase, with instructions for the use thereof to convert pyruvate and E4P to DAHP, and optionally with instructions for the conversion of said DAHP to at least
30 one derivative thereof.

41. The kit of Claim 40, wherein said cell is also capable of expressing at least one DHQ synthase.

42. The kit of Claim 41, wherein said cell is also capable of expressing at least one DHQ dehydratase.

43. A kit containing nucleic acid from which a cell can express at least one KDPGal aldolase, with instructions for the use thereof to transform a cell to produce a transformed cell that is capable of converting pyruvate and E4P to DAHP, and optionally to at least one derivative thereof.

5 44. The kit of Claim 43, wherein said kit contains nucleic acid from which a cell can express at least one DHQ synthase.

45. The kit of Claim 43, wherein said derivative of DAHP is at least one of DHQ, DHS, or a downstream derivative of DHS.

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